into knee joints at various time-points after induction of OA. OA phenotypes were measured within 8 weeks after induction. Total knee joints were isolated and processed for histology. Synovial activation was measured using an arbitrary scale (0 to 3) and cartilage destruction was measured in 4 different layers of the knee joint (medial and lateral tibia and femur) according to the scoring method of Pritzker et al.. Moreover, damage to the cruciate ligaments was scored using an arbitrary scale (0 to 5).

Results: After culture FACS analysis showed that the adherent fraction expressed characteristic markers for stem cells (positive staining for Sca-1, CD-44 and CD-105 and negative staining for CD-11b and cKIT). Using RT-PCR, we found that ASCs expressed high mRNA levels of TIMP 1, 2 and 3 but not TIMP 4. A single dose of ASCs (20×10^3in mouse serum) was injected into the knee joint of mice, 7 days after induction of osteoarthritis. Synovial activation was significantly inhibited at day 14 (9%) and day 42 (35%) when compared to serum treated joints. Destruction of cartilage was also significantly inhibited at day 14 (54%) and at day 42 (35%). Inhibition of cartilage destruction was particularly found in the medial tibia. Interestingly, ASC-treatment had a protective effect on the cruciate ligaments. At day 42, damage to the ligaments was reduced by nearly 50% in the ASC treated joints when compared to controls. In line with that, 87.5% of the control animals showed a dislocation of the knee joint, whereas only 25% of the ASC treated animals. In contrast to early treatment, injection of the same dose of ASCs, 14 days after induction of OA only showed a small inhibiting effect (11%) on synovial activation when measured at day 42. Although cartilage destruction diminished with 28%, these values did not reach significance at that time-point.

Conclusions: Our study indicates that a single injection of ASCs into the knee joints of mice with collagenase-induced osteoarthritis gives protection of synovial activation and cartilage destruction when given shortly (day 7) after induction of experimental OA, possibly by inhibiting MMP activity and protection of damage to cruciate ligaments.

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BETA-XYLOSIDES INHIBITION OF CHONDROITIN SULPHATE SUBSTITUTION ON MATRIX PROTEOGLYCANS PERTURBS THE DIFFERENTIATION OF BONE MARROW STEM CELLS INTO A CHONDROGENIC LINEAGE

S. Li, B. Caterson, A.J. Hayes, C.E. Hughes. *Sch. of BioSci.s, Cardiff Univ., Cardiff, United Kingdom*

Purpose: In a previous study (Hayes et al. J Histochem Cytochem. 2007, 56: 125–138) we reported that novel chondroitin sulphate (CS) sulphation motifs on cell-associated proteoglycans (PGs) may be putative biomarkers of progenitor/stem cell sub-populations (Dowthwaite et al. J Cell Sci. 2005, 117: 889–897). Recent studies indicate that unique CS sulphation motifs are localized in putative stem/progenitor cell niches at sites of incipient articular cartilage & other musculoskeletal tissues, which indicates their potential importance in cell differentiation during development. In this study, we investigated the importance of CS chains in the differentiation of bone marrow stem cells to the chondrogenic phenotype in vitro using p-nitrophenyl xyloside (PNPX) as a competitive inhibitor of CS substitution on matrix PGs.

Methods: Bovine bone marrow stem cells (BMSCs) were isolated from 7-day-old cow legs and cultured as monolayer for 4 weeks with chondrogenic medium ± 0.25mM PNPX. At each week, samples were analysed for sulphated glycosaminoglycans (sGAG), real-time PCR, Western Blotting & immunohistochemistry (IHC) using monoclonal antibodies recognising native and enzyme-generated epitopes & neoepitopes. The expression and distribution of structural chondroitin sulphation proteoglycans (CS-PGs) were analysed by immunofluorescent staining combined with confocal microscopy scanning.

Results: BMSCs cultured in chondrogenic medium start to aggregate and form mini-cell beads in 3 days and these mini cell beads clustered together to form a large cartilaginous cell bead in 2–4 weeks. There was no structural CS-PGs expression including aggrecan, biglycan & decorin when cells remained as monolayer. However, these CS-PGs were observed as long as the cells form mini-cell beads, suggesting an initiation of chondrogenic process. BMSCs cultured with 0.25mM PNPX still form the mini-cell beads and the single cartilaginous cell bead although it is 3–5 days later when compared with the control. This indicated that PNPX delayed the cell clustering and bead formation, a key milestone during the chondrogenic differentiation process. Real-time PCR and Western blotting results indicated that PNPX significantly inhibited or delayed the expression of chondrogenic markers such as aggrecan, SOX-9 & type II collagen gene and/or protein expression, suggesting the delay or inhibition of chondrogenic process. Interestingly, using monoclonal antibodies 7D4 and 6C3 identifying different epitopes along CS chains, we found that PNPX did not alter 7D4 staining but completely deplete the expression of 6C3 epitopes at the early stage of culture (1 week). Confocal microscopy analysis indicated that there was a colocalisation between 7D4 and aggrecan as well as biglycan core protein in the extracellular matrix of mini-cell bead after 2 weeks culture with or without PNPX. Differently, the colocalisation between 6C3 and biglycan was disrupted by PNPX, and there was no apparent colocalisation between 6C3 and aggrecan core protein. After 4 weeks of culture, 7D4 staining was observed across the cartilaginous-like bead and there was no difference between control and PNPX group. 6C3 staining was also evident across the whole bead in the control group. However, it was absent on the surface of the bead cultured with PNPX. Taken together, this highlight that different composition of CS chains may play distinct roles in the chondrogenic differentiation, and 6C3 epitopes are more important than 7D4 during bMSCs chondrogenesis.

Conclusion: These results indicated that CS sulphation motifs may play an important role in BMSCs differentiation into chondrogenic lineages. Its precise mechanism is not clear yet, but CS sulphation motifs may be involved in the cell aggregating, the initiation of chondrogenic process and extracellular matrix-cell interaction during the whole chondrogenesis.

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DOES ACOUSTIC HOMOGENEITY CORRELATE WITH TISSUE QUALITY IN ENGINEERED CARTILAGE?

J.F. Welter, M. Citak, H. Baskaran, A.I. Caplan, V.M. Goldberg, J.M. Mansour. *Case Western Reserve Univ., Cleveland, OH, USA*

Purpose: Osteoarthritis related cartilage defects affect a large and growing fraction of the population. As cartilage does not self-repair, these defects present a long term problem; the end-point is usually total joint replacement. Patching these defects with living tissue-engineered (TE) tissue, particularly based on mesenchymal stem cells (MSCs), is being considered as an approach to avoid or delay arthroplasty. In this approach, biocompatible scaffolds are seeded at high density with MSCs and then exposed to a chondroinductive medium *in vitro*. Despite uniform initial conditions, the engineered cartilage develops in a centripetal fashion. After several weeks in culture, there is a wide range of possible outcomes. The best case results in solid cartilage specimens being generated, the worst in a cartilage shell of varying thickness surrounding an immature soft center. From the outside, without resorting to histological evaluation, these extremes appear visually identical. This study is a proof-of-concept of the use of an inexpensive, non-imaging, ultrasound system to assess the internal homogeneity of TE cartilage samples by non-destructive testing.

Methods: Deidentified hMSCs were obtained from three healthy volunteer donors under the terms of an IRB approved protocol. The cells were culture-expanded until the end of 1st first passage and were then seeded at 8×10^8 cells/ml onto 6 mm diameter × 3 mm thick macroporous collagen-chondroitin sulfate scaffolds with different formulations. The cell-seeded constructs were then grown for up to 3 weeks in a perfusion bioreactor. In parallel, calf stifle joints were obtained from a local abattoir; the cartilage was shaved off and 6 mm diameter disks were then punched out using a biopsy punch. The normal cartilage disks or the engineered tissue were immersed in buffered saline. A Panametrics V116-RM ultrasound transducer was mounted in a custom jig and positioned at 90° to the surface of the test sample. A Panametrics 5072PR pulser-receiver and a Picoscope 3206 oscilloscope were used to capture the ultrasound data, after which the test samples were processed for histology.

Results: In normal bovine cartilage, clear acoustic reflections were obtained from the front and rear faces of the biopsy, and very few internal reflections were found. This correlates well with the homogeneous histological aspect of the native cartilage plugs. In the engineered tissue, in addition to the front and rear faces of the sample, numerous internal reflections of comparable magnitude could be found. Histological examination of the engineered tissue samples showed an irregular differentiated shell surrounding an undifferentiated cellular core. Thus,

while the native tissue was visually and acoustically homogeneous, the engineered tissue contains structural discontinuities which correlate well with the acoustic discontinuities.

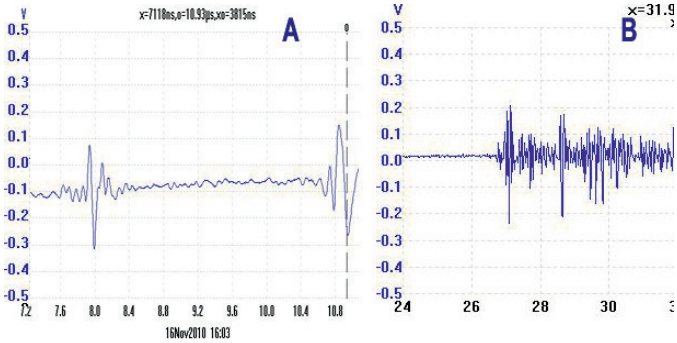

Fig. 1. Reflections from the front and rear faces of native bovine cartilage (A) or tissue engineered cartilage (B). In contrast to the native tissue, there are numerous reflections in the engineered tissue.

Conclusions: We have previously shown that speed of sound measurements can be used to estimate actual mechanical properties of hydrogels and are working to extend these findings to cartilage. The present proof of concept work suggests that ultrasound can also be used to assess, in a non-contact, non-destructive fashion the internal homogeneity of growing TE cartilage. We thus suggest that acoustic homogeneity of engineered tissue is a useful predictor of tissue homogeneity and thus can be used as a further quality control measure.

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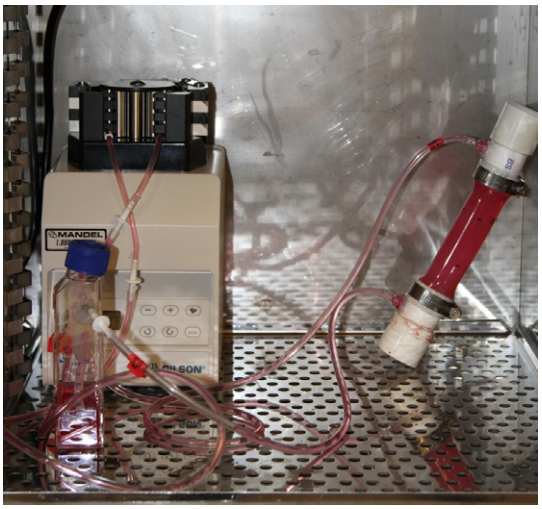
ENGINEERING MURINE STEM CELL DERIVED BONE AND CARTILAGE IN NOVEL BIOMIMETIC BIOREACTOR

P. Lee, D. Rancourt, R. Krawetz. *Univ. of Calgary, Calgary, AB, Canada*

Purpose: Research in bone and cartilage engineering for regenerative medicine approaches has expanded over the last decade; however, few methods have been clinically translated. Partially because most engineered constructs lack anatomical and mechanical characteristics of native tissues, and can be resorbed after implantation. In this study, we have derived an engineered bone and cartilage construct (EBCC) that is collagen based; within a novel biomimetic bioreactor. Previous results from our lab demonstrated collagen 1 and chondroitin sulphate to be potent inducers for osteogenesis and chondrogenesis respectively. However, prolonged maintenance cell viability and application of mechanical stimulants remains a hurdle in static tissue culture setting. Hence, a novel bioreactor system capable of emulating physiological environment specific for bone and cartilage development was designed; to enhance potential of the EBCC to possess both anatomical and mechanical resemblance to tissue in physiology.
Methods: A perfusion bioreactor was designed to provide continual flow of media to the EBCC at constant flow rate. The design allows alternation of media compositions in real-time, based on the needs of the EBCC at each stage of differentiation with minimal disruptions. An isolated polymerized collagen 1 platform was fabricated within the bioreactor to support embryonic stem cells (ESC) differentiation. Nutrients and oxygen gradients developed within the bioreactor are based on the design of bioreactor and seeding density of ESCs. This gradient is exploited (with media supplementation) to promote both osteogenesis and chondrogenesis at different parts of the platform; resulting in EBCC formation. Furthermore, the materials used to assemble the bioreactor are amenable to direct mechanical stimulation (bending and compression) and is applied to the developing EBCC to enhance differentiation.
Results: Cells in EBCC remained viable for at least 45 days post inoculation, with extensive cellular remodelling observed. A construct with firm structural features was developed. Quantitative PCR (qPCR) analyses demonstrated significant decrease in pluripotent marker, OCT-4, after 30 days, with coinciding increases in osteogenic and chondrogenic markers.
Conclusions: This novel bioreactor promotes long term cultivation and differentiation of the EBCC. Using the cast and mould concept, we had established an EBCC with specific shape, that retains mechanical properties after removal from the bioreactor. In future experiments we will be applying various mechanical stimulants on the EBCC to drive further maturation and transplant the construct into critical size skeletal defects. The EBCC principle, once validated in vitro and in vivo holds promises as an alternative approach for producing custom implants that can contain both bone and cartilage components.



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EFFECT OF SYNOVIAL FLUID ON THE IMMUNOMODULATORY CAPACITY OF MESENCHYMAL STEM CELLS

M.J. Leijs, G.M. van Buul, N. Kops, W.L. Koevoet, E. Lubberts, J.A. Verhaar, M.J. Hoogduijn, G.J. van Osch. *Erasmus MC, Rotterdam, Netherlands*

Purpose: Mesenchymal stem cells (MSCs) are promising candidates for cell therapeutic application in osteoarthritis (OA) and rheumatoid arthritis (RA). The catabolic environment in joints of OA and RA patients results in progressive joint damage. MSCs can have anti-catabolic or anti-inflammatory effects by secreting anti-inflammatory cytokines and growth factors. In order to become immunosuppressive MSCs need to be triggered by for instance pro-inflammatory cytokines. We hypothesized that synovial fluids influences the immunomodulatory effects of MSCs and investigated this in vitro by analyzing the gene expression of immunomodulatory factors.
Methods: Human bone marrow derived MSCs were cultured in monolayer in serum-free medium with 1% ITS with or without 20% synovial fluid for 48 hours. Synovial fluid of six OA donors, six RA donors and three healthy donors were used in triplicate cultures. Gene expression of immunoregulatory cytokines IL-6, HGF, TNFa, TGFb1 and